

7-1985

# The Antibody Production by Swine in Response to Sheep Red Blood Cells

Deborah Seymour

*Western Kentucky University*

Follow this and additional works at: <https://digitalcommons.wku.edu/theses>



Part of the [Agriculture Commons](#), and the [Meat Science Commons](#)

---

## Recommended Citation

Seymour, Deborah, "The Antibody Production by Swine in Response to Sheep Red Blood Cells" (1985). *Masters Theses & Specialist Projects*. Paper 2847.

<https://digitalcommons.wku.edu/theses/2847>

This Thesis is brought to you for free and open access by TopSCHOLAR®. It has been accepted for inclusion in Masters Theses & Specialist Projects by an authorized administrator of TopSCHOLAR®. For more information, please contact [topscholar@wku.edu](mailto:topscholar@wku.edu).

Seymour,

Deborah C.

1985



THE ANTIBODY PRODUCTION BY  
SWINE IN RESPONSE TO SHEEP RED  
BLOOD CELLS

A Thesis

Presented to

the Faculty of the Department of Agriculture  
Western Kentucky University  
Bowling Green, Kentucky

In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

by

Deborah C. Seymour

July 1985

## AUTHORIZATION FOR USE OF THESIS

Permission is hereby

☒ granted to the Western Kentucky University Library to make, or allow to be made photocopies, microfilm or other copies of this thesis for appropriate research or scholarly purposes.

☐ reserved to the author for the making of any copies of this thesis except for brief sections for research or scholarly purposes.

Signed Debbie L. Symons

Date July 25 '85

Please place an "X" in the appropriate box.

This form will be filed with the original of the thesis and will control future use of the thesis.



THE ANTIBODY PRODUCTION BY  
SWINE IN RESPONSE TO SHEEP RED  
BLOOD CELLS

Recommended July 11, 1985  
(Date)

Gordon F. Jones  
Director of Thesis

James A. Skean

Approved July 24, 1985  
(Date)

Colmer Gray  
Dean of the Graduate College

### ACKNOWLEDGEMENTS

I would like to thank Dr. Dan Skean, WKU Department of Biology, for all his technical assistance in this study in addition to his encouragement and support in my Master's Program.

I would also like to acknowledge the assistance and advice of Dr. Gordon Jones, WKU Department of Agriculture, who instigated this study and was instrumental in my participation in it.

I would also like to acknowledge the help and assistance of three undergraduate students without whom I could not have completed these experiments. To Tommy Maples, Elkmont, Alabama; Tom Sandifer, Jr., Bowman, South Carolina; and Todd Thieszen, Aurora, Nebraska; a heart-felt THANKS GUYS!



## TABLE OF CONTENTS

CHAPTER		PAGE
1	INTRODUCTION . . . . .	1
2	LITERATURE REVIEW . . . . .	4
3	METHODS AND MATERIALS . . . . .	9
	Experiment I - Determination of Site, Method of Injection and Correlation of Titer With Growth . . . .	10
	Experiment II - Determination of Dosage for Optimum Antibody Response . . . . .	11
	Statistical Analyses . . . . .	12
4	RESULTS AND DISCUSSION . . . . .	13
	Experiment I - Antibody Production . . . . .	13
	Growth Rate . . . . .	16
	Experiment II . . . . .	18
	SUMMARY . . . . .	22
	BIBLIOGRAPHY . . . . .	24

# LIST OF TABLES

TABLE		PAGE
1	Mean titer of anti-SRBC agglutinins in sera from groups of pigs given 2 injections of SRBC 4 weeks apart . . . . .	15
2	Coefficients of correlation between titers of antibody to SRBC in serum and weight gain in pigs given two injections of SRBC. Injections were given 4 weeks apart in two injection method treatments . . . . .	17
3	Coefficients of correlation between titers of antibody to SRBC in sera of pigs at weeks four, five and eight after treatments of 2 milliliters of 5% SRBC . . . . .	19
4	Comparison of natural logs of titer of antibody against SRBC in sera of pigs receiving two dosage-level treatments in two injections of SRBC eight weeks apart . . . . .	20



## LIST OF FIGURES

FIGURE		PAGE
1	Titers of anti-SRBC agglutinins in pigs given injections of 5% SRBC four weeks apart . . . . .	14
2	Titer of antibody against SRBC for two dosage level treatment groups of pigs receiving two injections of SRBC eight weeks apart . . . . .	21

THE ANTIBODY PRODUCTION BY  
SWINE IN RESPONSE TO SHEEP RED  
BLOOD CELLS

Deborah Carol Seymour

July 1985

25 pages

Directed by: Gordon F. Jones and J. Dan Skea

Department of Agriculture

Western Kentucky University

Two experiments were conducted to study the antibody response of pigs challenged with the general antigen sheep red blood cells (SRBC).

In one experiment SRBC's were injected at one of four sites: intramuscularly into the neck, intramuscularly into the ham, subcutaneously into the fore flank or rear flank. These treatments were repeated four weeks later. The antibody responses to the four treatments were determined by microtiter and analyzed statistically. No significant ( $P > 0.10$ ) differences were found among responses to the four treatments during the fourth and eighth weeks after injections, but a significant ( $P < 0.05$ ) difference was found during the fifth week among all four groups. Coefficients of correlation showed highly significant ( $P < 0.01$ ) relationships between SRBC antibody titer during the fourth week after injections and increase in weight of test animals from the beginning of the study until slaughter.

In a second experiment pigs were given intramuscularly either 2 milliliters of a 5% or 4 milliliters of a 10% SRBC suspension to determine the optimum dosage level. The difference between the antibody responses to these two treatments was not significant ( $P > 0.10$ ).

Results of this study indicate that when challenging hogs with SRBC, any of the commonly used sites for injection is acceptable. The injection of 2 milliliters of a 5% SRBC suspension is sufficient to



obtain antibody titers which differentiate among individuals. Coefficients of correlation indicate a relationship may exist between SRBC antibody titer and some economically important traits.

## CHAPTER 1

### INTRODUCTION

The use of subtherapeutic levels of antibiotics in animal feeds is a widespread practice among livestock producers today. Subtherapeutic levels are continued, low-level amounts of antibiotics, generally penicillin or tetracyclines, that are fed to animals in an effort to speed growth and improve feed efficiency. In the three years since antibiotics were first introduced as feed additives, their use has skyrocketed from about 265,000 pounds in 1951 to 2.3 million pounds in 1962 and 12.3 million pounds in 1978 (USDA 1980). Currently about half of all antibiotics produced are for agricultural use, with about 80% of that being feed additives (H.R. 7285).

Concern among consumers and government officials has risen over the development of strains of bacteria resistant to common antibiotics and the resulting long-term effects on human health. Therapeutic and subtherapeutic levels of antibiotics given directly to an individual may eventually increase the numbers of resistant bacteria in that individual. When the level of antibiotics administered is insufficient to kill all the bacteria, the bacteria remaining are those that are more resistant to that antibiotic. Eventually a population of resistant bacteria may be formed. The fear of those who are opposed to the use of subtherapeutic antibiotics is that the resistant bacteria may be transferred to humans through meat animals and thus alter the effectiveness of antibiotics used against human disease. If subtherapeutic antibiotics are administered to



a meat animal, bacteria residing in that animal may develop into resistant strains. If subsequently a resistant bacterium infects a human, the antibiotic therapy of choice may be ineffective. Thus a potential public health problem exists even though the occurrence of this chain of events has never been proven, particularly the transfer of resistant bacteria from animal to human.

A plausible alternative to subtherapeutic antibiotics in feed as a disease control measure is to select sows with strong inherent resistance to disease. A difference in disease resistance among sows and their offspring has been observed during disease outbreaks in confinement operations. Numerous factors could contribute to the ability of sows and their litters to resist disease. The sow's previous exposure to disease, body maintenance and the separate factors of the immune system itself may all be important, but it is difficult to assess the role these factors play in keeping an animal healthy. If it were possible to learn how genetic controls affect lymphocytes or antibody production, producers could use this knowledge to select for disease resistance in much the manner as for carcass traits and growth traits in their breeding stock. Producers already exercise control over exposure to disease and body maintenance.

Generally, some animals may be better prepared to deal with disease producing organisms because they are immunologically more competent than others. These animals are prepared to defend themselves from any type of invading foreign substance because their immune system adjusts quickly to form this defense.

It would be ideal to have a system for measuring response among a group of prospective herd replacements. If such a technique were developed

for measuring the immune response among animals, then the heritability of this response could be calculated. With heritability estimates, genetic improvement might be made for resistance to disease by selecting for breeding purposes those animals with the highest values for immunological response.

It has been proposed that within a group of hogs those exhibiting better antibody reaction to time general antigen would also be the individuals showing created disease resistance throughout their lives. These "healthier" hogs may grow faster and require less of the sub-therapeutic levels of antibiotic in feed to have good feed efficiency. Thus the animals with greater disease resistance should be more valuable as breeding stock.

The objectives of this study were five fold: (1) Determine the effectiveness of a general antigen for use in testing the hog's immune response. (2) Determine the optimum antigen dosage that would elicit a response but would not mask the differences among individuals. (3) The site for injection of the antigen and the method of injection were to be studied. (4) Determine the association, if any, between the rate of growth and the immune response. (5) Develop a technique for a fast, simple and accurate test of immune response. Thus, the test could later be used under field conditions with a minimal effort on the part of swine farmers and with no harmful effects on pigs.



## CHAPTER 2

### LITERATURE REVIEW

The idea that genetic controls may exist for some disease problems is not new. In poultry and in humans some specific diseases have been linked to genetic markers. In poultry, resistance to Marek's disease is linked to particular B alloalleles of the major histocompatibility complex. In a review on this tumor-causing disease, Briles (1977) cited studies revealing the differences in susceptible and resistant chicks. The B21 or resistant alloallele was a dominant trait over the B19 alloallele or susceptible alloallele. Chicks homozygous for the B21 alloallele were resistant while homozygous B19 chicks were eight times more likely to have the disease than were heterozygous B19 chicks. The alloallele type of chicks in question is found by typing the B blood group system. Alloalleles other than B21 and B19 also exist.

In humans, rheumatoid arthritis has been shown to be characterized by the presence of an altered antibody (Tizard, 1980) in the blood and a gene named HLA-DRw4 (Solinger, 1981). The gene, or a close neighbor, appears to cause the immune system to produce an altered immunoglobulin-G that attacks collagen, a protein important in holding bone joints together. The HLA-DRw4 gene is located in a region of the chromosome that is associated with rejection of foreign tissues.

In attempting to show how animals may possess general disease resistance, researchers have looked at several of the factors involved in an individual's general health. The inheritance of a serum protein is

found in swine, humans and cattle. A serum protein, designated protein B, is apparently controlled by a single pair of alleles and exhibits partial dominance in swine, according to Kristjansson (1960). BB genotypes appear to have twice the amount of protein B as do Bb genotypes, while bb genotypes show no evidence of the protein. When serum samples from 100 litters were analyzed for genotype and compared to the genotype of their parents, the data suggested a semi-lethal interaction between a Bb genotype progeny and a BB genotype dam. This study shows that blood serum components are indeed heritable and traceable.

Rasmusen (1960) concluded that although it is not likely that single genes such as those controlling blood groups, have important effects on economic traits, they might provide useful genetic markers.

Jensen et al. (1968) tested 16,000 pigs (Durocs and Hampshires) for phenotype in twelve blood group systems and four serum protein systems and measured ten performance traits of economic importance. They reported that different types of one blood group system, the H red cell antigens, had extremely significant effects for several traits in Durocs and some significant effects in Hampshires. Of the alloalleles a and c, homozygous H(c/c) sows weaned about one more pig per litter than did homozygous H(a/a) sows with the heterozygous H(a/c) sows intermediate between the two. Litter weight at 54 days of age showed significant advantages in the H(a/a) animals. This appears to be inherited dominantly with H(a) over H(c). Rasmusen (1980) theorizes that the H type may have its effect on growth traits because the H locus may be in a region of the chromosome close to the genes which are involved in energy metabolism. In addition, the genes for reproduction may be very close to the H locus.



Rothschild (1982) suggests that Swine Lymphocyte Antigens (SLA) may be important genetic markers for immune response genes. The genes for the SLA group are located very close on the chromosome to those genes controlling the immune response.

In studies on swine humoral immunity, several different antigens have been used in testing procedures. Brown et al. (1961) conducted five experiments studying the colostrum-acquired immunity and active antibody production in baby pigs. Serratia marcescens was used in four of the experiments to "investigate the effect of weaning pigs at two weeks of age on the persistence of colostrum-acquired immunity, (and) the effect of colostrum-acquired immunity on active antibody production. . . ." S. marcescens was selected by the group for use because it is not normally found in the animal's body and would not grow at 37° Celsius, the hog's normal body temperature. Blecha and Kelley (1981) evaluated the effects of stress on the antibody-mediated immune response of the pigs by using sheep red blood cells (SRBC) as an antigen. Five milliliters of the 40% SRBC suspension were injected at 345 days of age and it was determined that the multiple stressors of cold and weaning did not produce an interactive effect. The stressors of cold and weaning produced surprising results: cold stress enhanced antibody production in greater proportion than weaning stress depressed production. Haye and Kornegay (1979) investigated the antibody response of sow-reared and artificially-reared pigs using a 10% SRBC suspension and salmonella H antigen. Both were given at the rate of one milliliter of antigen suspension per 100 milliliters of estimated blood volume. The authors concluded that the artificial rearing system had no major effects on immunoglobulin synthesis. Huang et al. (1981) reported using one milliliter of 10% SRBC suspension

in an experiment designed to "establish a selective breeding herd of immunological responsibility." The Taiwanese researchers found very low correlations between antibody titers to SRBC and the economic traits exhibited by the test animals. Phenotypic correlations between primary and secondary antibody titers were positive.

The methods of injection of antigen differed among researchers as did the method of acquiring blood samples. Hendrix et al. (1978) obtained blood samples from twelve-hour-old piglets via the orbital sinus when exploring porcine neonatal serum gamma globulins. Blecha and Kelley et al. (1981) also used the orbital sinus method of bleeding and intravenously injected the antigen. W.G. Pond (1980) states that as much as a ten milliliter blood sample may be obtained via the orbital sinus on 200-pound hogs. A ten-milliliter pipet is used to obtain the sample. Brown et al. (1961) used anterior vena cava puncture to get blood samples and intravenously injected the antigen.

Haye and Kornegay (1979) found that pigs less than fifteen days of age failed to respond to SRBC immunization. It has been well established that the piglet is born essentially devoid of circulating antibodies, and the immune system of a piglet does not begin functioning prior to two weeks of age (Pond, 1980). Brown et al. (1961) studied colostrum-acquired immunity and injected antigen into sows. Huang et al. (1981) used 120-day-old pigs and reported finding maximum titers within one week after the primary injection was administered.

The use of subtherapeutic levels of antibiotics is not limited to swine, nor is it a small problem. Current estimates show that 100% of all poultry raised in this country receive antibiotics in subtherapeutic levels, some 90% of swine and veal and 60% of the cattle receive similar



levels of antibiotics (H.R. 7285).

Chase Econometrics estimated that if a total ban of penicillins and tetracyclines were put into effect, during the first year following enactment there would be a consumer price increase of 1.3 to 3.7% (H.R. 7285).

Therefore, while the problem of excess antibiotic use exists, the technology and knowledge necessary to solve the problem also exists.

### CHAPTER 3

#### METHODS AND MATERIALS

Sheep Red Blood Cells (SRBC) were selected as the general antigen for use in the research for three reasons: (1) SRBC's would not normally be found in the pig's environment and thus there would be practically no chance of its already possessing antibodies for SRBC, (2) SRBC's are available in a standard suspension from several biological supply houses and therefore would be convenient to use, (American Scientific Products, Atlanta, Georgia), and (3) SRBC's are nonpathogenic and would not pose a health threat to the remainder of the hogs in the herd.

Blood samples were taken via the anterior vena cava with a 16-gauge, 1.5-inch needle on a 10 milliliter syringe, transferred into screwcapped centrifuge tubes and allowed to clot at ambient temperature.

Serum samples were obtained by centrifuging the tubes of clotted blood in an International Equipment Centrifuge (Model No. HL) for ten minutes at approximately 5000 rpm, then the supernatant serum was harvested into a 15-cubic centimeter test tube with a Pasteur pipet and bulb. Then these were centrifuged again for five minutes to remove residual cells and harvested into another 15 cubic-centimeter test tube and held in a hot water bath at 37° Celsius for 30 minutes to inactivate the complement. Samples were sealed in the test tubes with Parafilm and frozen until assayed for antibody.

Antibody to SRBC in each serum was determined by microtiter hemagglutination assay in standard 8-X 12-well, U-bottom plates (Cooke



Laboratory Products, Alexandria, Virginia). Serum samples of 25 microliters were diluted serially in 25 microliters of physiological saline. Twenty-five microliters of 10% SRBC suspension were added to each well of the microtiter plate. The plates were incubated at 25<sup>o</sup> Celsius for one hour, then refrigerated undisturbed for 16-18 hours before being evaluated. Antibody titer was recorded as the reciprocal of the highest dilution in which visible agglutination had occurred. All samples were assayed in duplicate and the results were considered valid if the agglutination end-points of duplicates were within one well or one dilution of each other.

#### Experiment I Determination of Site, Method of Injection and Correlation of Titer With Growth

Twenty weaned pigs were selected at random from the nursery at the Western Kentucky University Swine Unit. The pigs, various crosses of Hampshire, Landrace and Yorkshire, were between 34 and 48 days of age at the beginning of the study. Individual ear notches were recorded and preliminary blood samples were taken; thereafter blood samples were taken at seven-day intervals for twelve weeks. Pigs were weighed and the individual weights recorded. Then they were randomly assigned to one of four treatment groups designated as follows: IM, intramuscular injection into the neck; IMH, intramuscular injection into the ham; SFF, subcutaneous injection into the fore flank; and SRF, subcutaneous injection into the rear flank. Five pigs were used in each treatment group and each received 2 milliliters of 5% SRBC at the site indicated for that group. This treatment was repeated five weeks later.

Prior to the beginning of the study, the pigs had been grouped by weight into three elevated, 4' x 4' nursery pens at weaning. Pigs

being tested were selected at random from each pen when the experiment began and received the same management as their penmates not on test. Approximately four weeks after the first injection, all pigs were moved into four grower pens on concrete slats that were located in the same room as the nursery pens. Pigs were again segregated by weight with no distinction made among treatment groups and pigs not on test. All pigs were fed ad libitum a 16% protein, corn-based diet fortified with adequate vitamins and minerals.

Approximately eight weeks after the first injection was given, all hogs were moved from the grower pens to open-front concrete finishing units with straw bedding. There they received ad libitum water and a 14%-protein, corn-based diet fortified with adequate vitamins and minerals. Prior to shipment for slaughter, hogs were again individually weighed to determine the amount of growth during the testing period.

#### Experiment II Determination of Dosage for Optimum Antibody Response

Twenty four pigs from four litters between 51 and 69 days of age, ear-notched for identification, were included in treatment groups as follows: T-1, two from each litter received 2 milliliters of 5% SRBC suspension; T-4, two from each litter received 4 milliliters of 5% SRBC suspension; T-01, one from each litter received 2 milliliters of physiological saline; and T-04, one from each litter received 4 milliliters of physiological saline.

Ear notch identification was recorded and preliminary blood samples were taken. Each treatment was given as an intramuscular injection into the neck area. A blood sample was taken 14 days after the first injection was given and at seven-day intervals thereafter for 12 weeks.



To stimulate the secondary antibody response, seven weeks after the first injection pigs received a second injection identical to the first.

Approximately two weeks after being given the first injection, pigs were moved from nursery decks to grower pens as in Experiment I. Four weeks later they were moved to the finishing unit and maintained there as in Experiment I.

#### Statistical Analyses

The analysis of variance and of the coefficients of linear correlation were calculated as suggested by Little and Hill (1978). Correlations and F values were tested for significance at the 0.01, 0.05, and 0.10 levels.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### Experiment I Antibody Production

The number of individuals exhibiting each different titer is shown in Figure 1. A line on the graph illustrates the median titer for that week. Weeks four, five and eight were chosen for statistical analysis. Week four is representative of a primary reaction while five is representative of the peak of a secondary response. Week eight was chosen to represent the waning of a secondary response. Means of titers for each treatment at four, five and eight weeks post-treatment are given in Table 1.

##### Week 4

No significant ( $P > 0.10$ ) differences among the treatments were observed.

##### Week 5

After blood samples were drawn at the fourth week post-treatment, a second SRBC treatment was administered, thus week five titers probably reflect some secondary immune response. However, titers obtained during this experiment may not be a true illustration of a secondary reaction. A rigorous demonstration of a secondary response would require allowing the median primary response curve (Figure 1) to decline to a minimal level. This was not done, and thus the means of titers for the fifth week following the first treatment are probably



Figure 1. Titers of anti-SRBC agglutinins in pigs given injections of 5% SRBC four weeks apart. Values shown in bar graphs represent the number of pigs with that titer to SRBC.

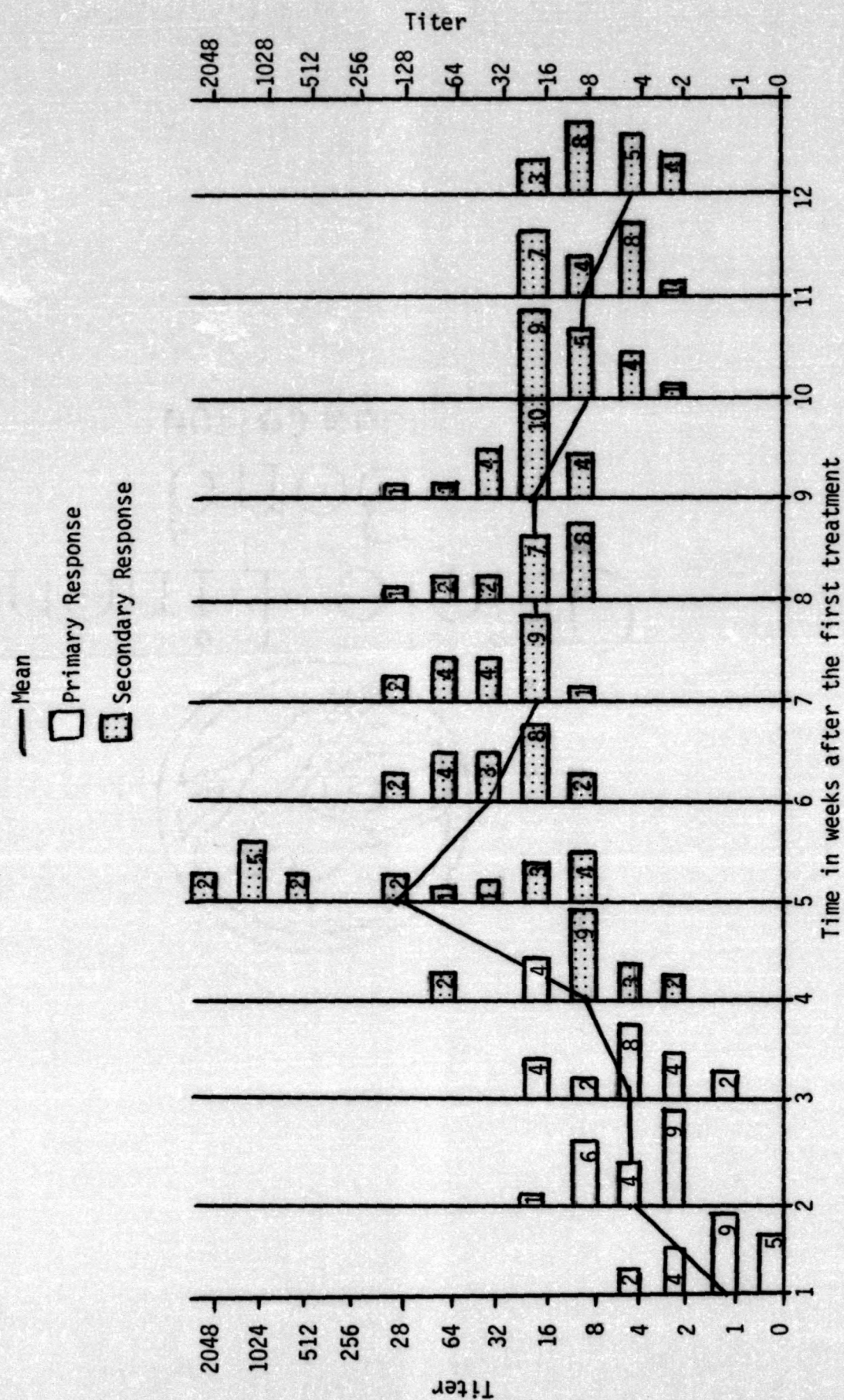


Table 1. Mean titer<sup>a</sup> of anti-SRBC agglutinins in sera from groups<sup>b</sup> of pigs given 2 injections<sup>c</sup> of SRBC 4 weeks apart.

Injection group	Weeks after first injection			
	0	4	5	8
IMN <sup>d</sup>	0	17.2 h	43.2 i	11.2 n
IMH <sup>e</sup>	0	7.6 h	1028.8 j	22.4 n
SFF <sup>f</sup>	0	11.2 h	819.2 k	19.2 n
SRF <sup>g</sup>	0	20.0 h	243.2 m	46.4 n

<sup>a</sup>Determined by a microagglutination technique. Those followed by the same letter are not significantly ( $P > 0.10$ ) different; those followed by different letters are significantly ( $P < 0.01$ ) different.

<sup>b</sup>Five pigs per group.

<sup>c</sup>Each was 2 ml of 5% suspension of SRBC in aqueous 0.9% NaCl.

<sup>d</sup>Intramuscular in neck.

<sup>e</sup>Intramuscular in ham.

<sup>f</sup>Subcutaneous in fore flank.

<sup>g</sup>Subcutaneous in rear flank.



not what would be expected from a secondary reaction. Duncan's multiple range test showed that all four treatment means were significantly ( $P < 0.05$ ) different.

#### Week 8

Mean titers had begun decreasing to a minimal level by the eighth week following the first treatment and were not significantly ( $P > 0.10$ ) different among the treatment groups.

#### Growth Rate

The coefficients of correlation between growth rate and anti-SRBC titers calculated for weeks four, five, and eight following the first treatment are presented in Table 2. Although there were not enough individuals on test to assure an accurate estimate, the trends of the coefficients of correlation indicate that further study is merited.

#### Week 4

All correlations were significant ( $r_{0.01} = .56140$ ) (Table 2). This is not in agreement with Huang et al. (1981), who found no correlation between SRBC antibody titer and economic traits.

#### Week 5

Only the correlation for subcutaneous treatments was significant ( $r_{0.01} = .5614$ ). It is interesting to note that week five following the first treatment was the only time during which there was a significant difference among means of antibody titers was obtained from the different treatments (Table 1).

#### Week 8

None of the correlations between the week 8 antibody titer to SRBC and weight gain were significant.

Table 2. Coefficients of correlation between titers of antibody to SRBC in serum and weight gain<sup>a</sup> in pigs given two injections of SRBC<sup>b</sup>. Injections were given 4 weeks apart in two injection method treatments<sup>c</sup>.

	Week after first treatment		
	4	5	8
All animals <sup>d</sup>	.578	.180	.079
IM treatments only <sup>c</sup>	.675	.136	-0.129
SC treatments only <sup>c</sup>	.577	.570	.195

$r_{.10} = .3783$

$r_{.05} = .4438$

$r_{.01} = .5614$

<sup>a</sup>Gain in weight from beginning of study until slaughter.

<sup>b</sup>Two milliliters of 5% SRBC in aqueous solution of NaCl.

<sup>c</sup>Intramuscular injections in the ham and neck. Subcutaneous injections in the fore flank and the rear flank.

<sup>d</sup>Twenty pigs total.



The coefficients of correlation between change in weight and antibody titer appear to decline during the secondary reaction. The correlation between antibody titers of each week also declined (Table 3). This may indicate that the level or magnitude of the secondary response to antigens may be independent of that of the primary response.

### Experiment II

The means of natural logs of the antibody titers for the two treatments at weeks five, six, eleven, and twelve are given in Table 4. There was no significant ( $P > 0.10$ ) difference between these two means at any test week.

By graphing the antibody titer versus time (twelve weeks of blood tests) and showing the number of animals with each titer as a bar graph, a curve of the median titer for each treatment is illustrated (Figure 2).

The resulting curves were not as expected. The T-1 curve more closely resembles a typical antibody response curve and peaks in both the primary and secondary reactions at one dilution higher than does the T-4 treatment.

The T-1 and T-4 curves both decline to the same titer. In addition, the T-4 curve takes an unexplained decline in the third week following the first treatment (Figure 2). Management procedures were the same for all four litters involved in the experiment and any stress occurring should have affected equally the pigs in both treatment groups.

In both Experiment 1 and Experiment 2 the maximum primary antibody titers occurred about five weeks after the first injection. This is much later than that reported by Huang et al. (1981).

Table 3. Coefficients of correlation between titers of antibody to SRBC in sera of pigs at weeks four, five and eight after treatments of 2 milliliters of 5% SRBC.

	Week after first treatment		
	4	5	6
Week 4			
All animals <sup>a</sup>		.288	.096
IM only <sup>b,c</sup>		.157	.013
SC only <sup>b,d</sup>		.360	.152
Week 5			
All animals <sup>a</sup>	.288		.186
IM only <sup>b,c</sup>	.157		.452
SC only <sup>b,d</sup>	.360		-0.198

$r_{.10} = .3783$

$r_{.05} = .4438$

$r_{.01} = .5614$

<sup>a</sup>Twenty pigs total.

<sup>b</sup>Ten pigs total.

<sup>c</sup>Intramuscular injections in ham or neck.

<sup>d</sup>Subcutaneous injections in fore flank or rear flank.



Table 4. Comparison of natural logs of titer of antibody against SRBC in sera of pigs receiving two dosage-level treatments<sup>a</sup> in two injections of SRBC eight weeks apart<sup>b</sup>.

Week after first injection	Treatment Group	
	T-1	T-2
5	1.21300	1.47294
6	1.03972	1.29965
11	4.07284	4.67876
12	3.55238	3.79636

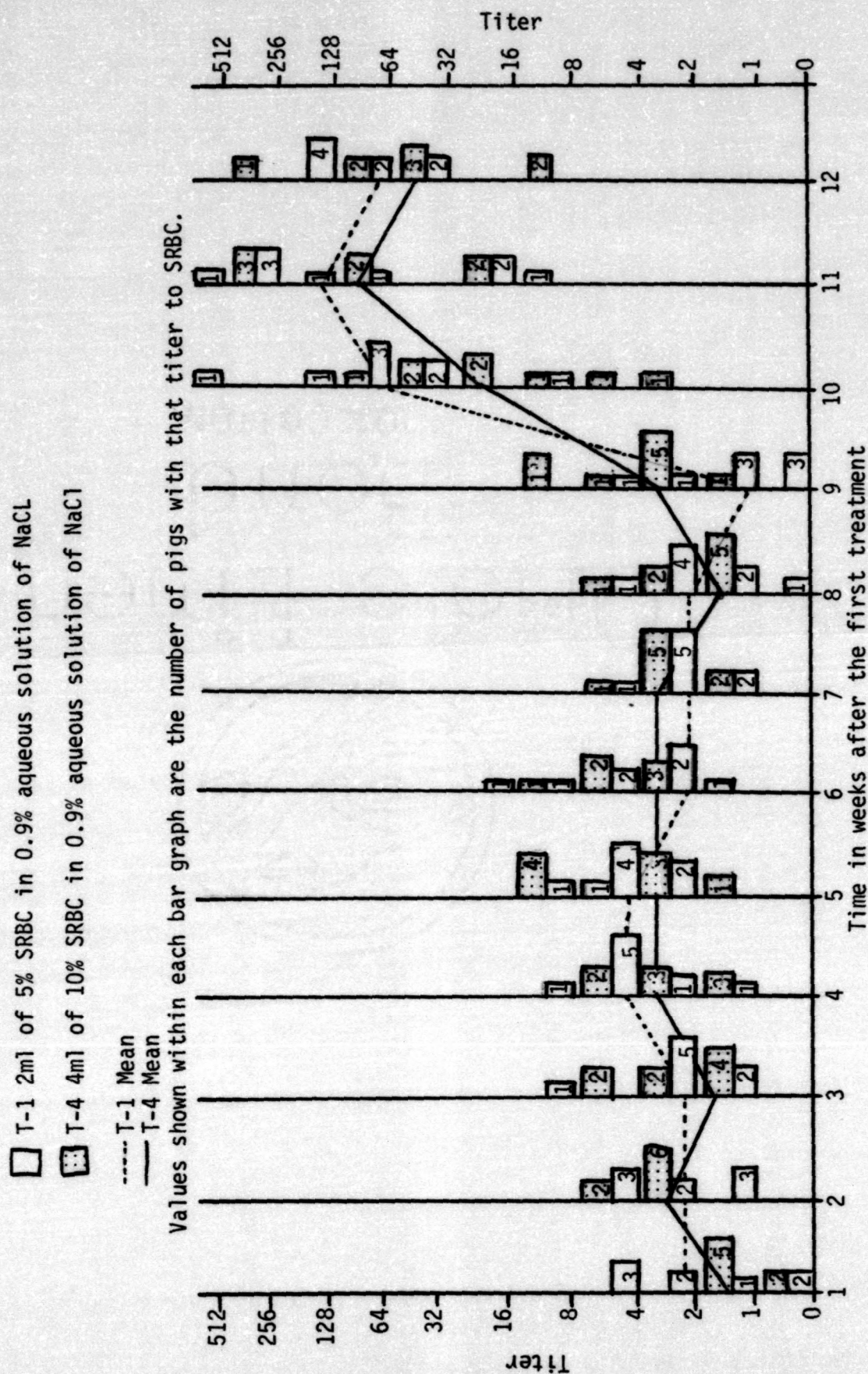
None of the values were significant at either the 0.05 or 0.01 level.

<sup>a</sup>T-1 received 2 ml 5% SRBC in 0.9% aqueous solution in NaCl.

T-2 received 4 ml 10% SRBC in 0.9% aqueous solution in NaCl.

<sup>b</sup>Eight pigs per group represented.

Figure 2. Titer of antibody against SRBC for two dosage-level treatment groups of pigs receiving two injections of SRBC eight weeks apart. Median values are shown for each treatment group.





## SUMMARY

Pigs from the Western Kentucky University Swine Unit and laboratory facilities of the WKU Department of Agriculture and Department of Biology were utilized in a study of the antibody produced in response to injections of Sheep Red Blood Cells. The study was conducted from September 1981 to March 1982.

In Experiment I, 20 pigs received a general antigen, Sheep Red Blood Cells (SRBC) in one of four locations: IMN, intramuscular injection in the neck; IMH, intramuscular injection in the ham; SFF, subcutaneous injection in the fore flank; or SRF, subcutaneous injection in the rear flank.

Only during the fifth week post-treatment was there any significant ( $P < 0.01$ ) differences among treatments, the IMH treatment showing the highest antibody titer. These results indicate that antibody production is not generally influenced by the site of antigen injection.

There was a highly significant ( $P < 0.01$ ) correlation between antibody titer at week four post treatment and the amount of weight the pig gained from the beginning of the experiment until slaughter. There was no association between antibody titer at weeks five or eight post-treatment and total weight gained.

While there were not enough pigs in the study to yield sufficient data from which to draw conclusions concerning the relationship between antibody response to a general antigen and weight gain, the statistics indicate further studies are warranted.

In Experiment II, two dosage levels of SRBC were compared: T-1, 2 milliliters of 5% SRBC suspension; and T-4, 4 milliliters of 10% SRBC suspension. No differences ( $P > 0.10$ ) were found between treatments during weeks five, six, eleven and twelve following the first injection. However, the median curve of antibody production for each treatment during the experiment indicated maximum production occurred in both primary and secondary reactions with the T-1, or smaller amount of antigen.

For conducting further studies, results of these experiments show:  $\times$

(1) SRBC is an effective general antigen for use in determining an animal's immunocompetence, (2) From results in Experiment II, 2 milliliters of 5% SRBC suspension appears to be a sufficiently large amount of antigen to differentiate between individual responses, (3) The injection site for SRBC is not important and in Experiment II, IMN was used for convenience and because potential users of this kind of test would be more familiar with giving an intramuscular injection. In addition, there would be less chance of damaging a valuable market cut, (4) Preliminary results indicate good correlations exist between SRBC antibody titer and weight gain, an economically important trait.



## BIBLIOGRAPHY

- Carle, B.H. and W.H. Dewhirst, Jr. 1942. A method for bleeding swine. *Journal of Animal Science*. 101:495.
- Blecha, Frank and Keith W. Kelley. 1981. Effects of Cold and Weaning Stressors on the Antibody-Mediated Immune Response of Pigs. *Journal of Animal Science*. 53:439.
- Briles, W. Elwood, Howard A. Stone and R.K. Cole. 1977. Marek's Disease: Effects of B Histocompatibility Alloalleles in Resistant and Susceptible Chicken Lines. *Science*. 195:193.
- Brown, Herbert, V.C. Speer, L.Y. Quinn, V.W. Hays and D.V. Catron. 1961. Studies on Colostrum-Acquired Immunity and Active Antibody Production in Baby Pigs. *Journal of Animal Science*. 20:323.
- Haye, S.N. and E.T. Kornegay. 1979. Immune Response in Sow- and Artificially-Reared Pigs. *Journal of Animal Science*. 48:1116.
- Hendrix, William, Keith W. Kelley, Charles T. Gaskins, and David J. Hunrichs. 1978. Porcine Neonatal Survival and Serum Gamma Globulins. *Journal of Animal Science*. 47:1281.
- Huang, Jiin-Yuan, C.N. Weng, Y.T. Huang, T.L. Young. 1981. Personal Letter from Senior Author and Summaries of Reports of Animal Industry Research Institute, Taiwan Sugar Corp., Chunan, Taiwan, ROC.
- H.R. 7285. Hearing before Subcommittee on Health and Environment of the Committee on Interstate and Foreign Commerce. United States House of Representatives, United States Congress. Serial Number 96-169.
- Jensen, E.L., C. Smith, L.N. Baker and D.F. Cox. 1968. Quantitative Studies on Blood Group and Serum Protein Systems in Pigs. II. Effects on Production and Reproduction. *Journal of Animal Science*. 27:856.
- Little, Thomas M. and F. Jackson Hills. 1978. *Agricultural Experimentation, Design and Analysis*. John Wiley and Sons Publishers, New York, New York.
- Kristjansson, Fred K. 1960. Inheritance of a Serum Protein in Swine. *Science*. 188:1981.
- Pond, W.G. and K.A. Houpt. 1979. *Biology of the Pig*. Cornell University Press, Ithaca, New York.

Rasmusen, B.A. 1981. Blood Groups and Pork Production. BioScience. 31:512.

Solinger, Alan M., Rajendra Bhatnagar and John D. Stobo. 1981.  
(Abstract) Arthritis: Sabotage by the Immune System? Science  
News. 120:186.

USDA, Economics and Statistics Service. 1980. FARMLINE. Antibiotic  
Feed Additives: Doing Without. Vol. I, No. 9, p. 14.



B5, F13